UNITED STATES ENVIRONMENTAL PROTECTION AGENCY
WASHINGTON, DC 20460

OFFICE OF CHEMICAL SAFETY
AND POLLUTION PREVENTION

August 26, 2013

MEMORANDUM

SUBJECT: Efficacy Review for Hitman Wipe
EPA Reg. No. 9402-RT
DP Barcode: 411421

FROM: Karen Hill, Ph.D.
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K. Hill 8/26/13

THRU: Mark Perry
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MP

TO: Velma Noble RM31/ Druscilla Copeland
Regulatory Management Branch I
Antimicrobials Division (7510P)

APPLICANT: Kimberly-Clark Professional
1400 Holcomb Road
Roswell, GA 30076-2199

FORMULATION FROM LABEL:

Active Ingredient(s)	% by wt.
Hydrogen Peroxide	0.94%
Didecyldimethyl ammonium carbonate (and) Didecyldimethyl ammonium bicarbonate	1.25%
Inert Ingredients	97.81%
Total	100.00%

I. BACKGROUND:

The registrant is seeking a new registration for the product Hitman Wipe as a towelette with disinfectant, non-food contact sanitizer, and residual self-sanitizer claims for use on hard non-porous surfaces in residential and non-healthcare institutional and industrial settings. Studies were conducted by ATS Labs, 1285 Corporate Center Drive, Suite 110, Eagan, MN 55121 and Antimicrobial Test Laboratories, 1304 West Industrial Blvd., Round Rock, Texas 78681. Examination of the concentration of active ingredients within the batches tested was performed by Case Laboratories, Inc. 622 Route Ten Whippany, NJ. 07981.

The data package included a cover letter, Certificate with Respect to Citation of Data (EPA Form 8570-34), Formulator's Exemption Statement (EPA Form 8570-27), Data Matrix (EPA Form 8570-35), a proposed product label, transmittal documents, and fourteen efficacy studies (MRID 490899-08 through -21) with a Statement of No Data Confidentiality Claims embedded within each MRID.

II. USE DIRECTIONS:

The product, Hitman Wipe, is intended to be used as a disinfectant and sanitizer on hard, non-porous, non-food contact surfaces including appliance exteriors, chairs, counters, doorknob, faucets, trash cans, hampers, sinks, vinyl shower curtains, toilet seats, tables, home gym equipment, and laminate countertops.

The label provides the following use directions:

To Sanitize [Non-Food Contact Sanitizer] [deodorize]:
For hard, non-porous, non-food contact surfaces [{or:}To sanitize hard, non-porous, non-food contact surfaces.] Wipe surface using enough wipes to allow treated surface to remain wet for [15 seconds]. [Let surface dry][air dry]. No rinsing required.] Remove heavy soil prior to sanitization.

To Clean and Disinfect:
To disinfect hard, non-porous surfaces, clean surface to be treated using a wipe to completely remove all debris [soil]. Use a fresh [new] wipe to disinfect, thoroughly wetting surface to be treated. Use enough wipes for treated surfaces to remain visibly wet for six [6] minutes. [No rinsing required.] [When used as directed, Hitman Wipe provides residual protection [from *Staphylococcus aureus*, *Enterobacter aerogenes*, and Community-Associated Methicillin Resistant *Staphylococcus aureus* (CA-MSRA)] for up to 24 hours.]

Residual-Self Sanitizing:
To sanitize for 24 hours [against] [bacteria] [including] *Staphylococcus aureus*, *Enterobacter aerogenes*, and Community-Associated Methicillin Resistant *Staphylococcus aureus* (CA-MSRA) on hard, non-porous, non-food contact surfaces. Use enough wipes to thoroughly [uniformly] treat surfaces [to remain visibly wet for five [5] minutes. [Air dry.] [No rinsing required.] [Provides residual sanitizing activity for up to 24 hours.] This product can be removed with soap and water. [If soap and water is used,] repeat residual self-sanitizing directions to maintain 24 hour sanitization.

To inhibit [Control] Mold and Mildew:
For use on hard, non-porous surfaces. Clean surface first, then treat [wipe] surface, using

enough wipes to thoroughly [uniformly] wet the surface. Allow to air dry, reapply weekly.

III. AGENCY STANDARDS:

Antimicrobial Products for Use on Hard Surfaces Using Pre-saturated or Impregnated Towelettes:

Towelette products represent a unique combination of antimicrobial chemical and applicator, pre-packaged as a unit in fixed proportions. As such, the complete product, as offered for sale, should be tested according to the directions for use to ensure the product's effectiveness in treating hard surfaces. The standard test methods available for hard surface disinfectants and sanitizers, if followed exactly, would not closely simulate the way a towelette product is used. Agency guidelines recommend that a simulated-use test be conducted by modifying the standard test methods. Agency guidelines further recommend that instead of spraying the inoculated surface of the carrier, the product should be tested by wiping the surface of the carrier with the saturated towelette, and then subculturing the slides after a specified holding time. Sixty carriers must be tested with each of 3 product samples, representing 3 different product lots, one of which is at least 60 days old. The towelette should be removed from its container and subsequently handled with sterile gloves. One towelette should be used to wipe at least 10 inoculated slides. To support products labeled as "disinfectants," killing on 59 out of 60 carriers is required to provide effectiveness at the 95% confidence level. One carrier with a surface area equivalent to ten 1 x 1 inch carriers or ten carriers each with a surface area of 1 x 1 inch should be wiped using one towelette per carrier set (for a total of six towelettes and 60 carriers) per batch. The area of the towelette used for wiping should be rotated so as to expose a maximum amount of its surface in the course of wiping a set of slides. A detailed description of the wiping procedure, including the towelette folding and rotation process should be included in the test protocol and documented in the raw data and final report. The applicant should submit their towelette protocol to the Agency for review and approval prior to conducting the test.

Disinfectants for Use on Hard Surfaces (Against a Broad Spectrum of Bacteria):

The effectiveness of disinfectants for use on hard surfaces must be substantiated by data derived using the AOAC Use-Dilution Method (for water soluble powders and liquid products), AOAC Hard Surface Carrier Test (for water soluble powders and liquid products), or the AOAC Germicidal Spray Products as Disinfectants Method (for spray products). Sixty carriers must be tested with each of 3 product samples, representing 3 different product lots, one of which is at least 60 days old, against a mean log density of at least 6 for *Staphylococcus aureus* (ATCC 6538). To support products labeled as "general disinfectants" for all the test methods listed above killing on 59 out of 60 carriers is required to provide effectiveness at the 95% confidence level except for AOAC Hard Surface Carrier test where killing on 58 out of 60 carriers is required to provide effectiveness at the 95% confidence level and the dried carrier count is to be 0.5 – 2.0 X 10^6 for *Salmonella enterica* and 1 – 5 X 10^6 for *Staphylococcus aureus*.

Disinfectants for Use as Fungicides (Against Pathogenic Fungi):

Effectiveness of liquid disinfectants against specific pathogenic fungi must be supported by efficacy data derived from each of 2 samples representing 2 different batches using the AOAC Fungicidal Test. The test should be conducted at 5, 10, and 15 minute exposure times. Alternatively, the AOAC Use Dilution Method, modified to conform with appropriate elements in the AOAC Fungicidal Test, may be employed. If the product is intended for use as a spray, the AOAC Germicidal Spray Products Test must be employed. Performance requirements for this test: the highest dilution that kills all fungal spores is the minimum effective concentration. Ten

carriers for each of two samples representing two different batches of the product should be evaluated against *Trichophyton mentagrophytes* (ATCC 9533). The inoculum employed should provide a concentration of $1 \times 10^4 - 1 \times 10^5$ conidia per carrier. For the AOAC International Fungicidal Activity of Disinfectants test, all fungal spores at 10 and 15 minutes should be killed to support a 10 minute exposure time. For the AOAC International Use-Dilution Methods, all fungal spores on all 10 carriers should be killed in ≤ten minutes.

Sanitizers (For Non-Food Contact Surfaces)

The effectiveness of sanitizers for non-food contact surfaces must be supported by data that show that the product will substantially reduce the numbers of test bacteria on a treated surface. The test surface(s) should represent the type(s) of surfaces recommended for treatment on the label, i.e., porous or non-porous. Products that are represented as "one-step sanitizers" should be tested with an appropriate organic soil load, such as 5 percent serum. Tests should be performed with each of 3 product samples, representing 3 different product lots, one of which is at least 60 days old against *Staphylococcus aureus* (ATCC 6538) and either *Klebsiella pneumoniae* (aberrant, ATCC 4352) or *Enterobacter aerogenes* (ATCC 13048). Results must show a bacterial reduction of at least 99.9 percent over the parallel control within 5 minutes.

Residual Self-Sanitizing Products:

The effectiveness of sanitizers that bear claims of residual activity must be supported by data that show that the product continues to reduce the number of challenge microorganisms over an identified period of time. Products with residual self-sanitizing activity keyed to the presence of moisture on surfaces should be tested in a controlled or simulated in-use study. The study should be designed in consultation with the Agency. Products with residual self-sanitizing activity intended for use on dry surfaces should be tested in accordance with Protocol #01-1A, Protocol for Residual Self-Sanitizing Activity of Dried Chemical Residues on Hard, Non-Porous Surfaces. These Agency standards are presented in OPPTS 810.2100. EPA Protocol #01-1A does not specify the number of product lots or the organisms that must be tested; however, DIS/TSS-10 standards for sanitizers for non-food contact surfaces require testing of 3 product samples, representing 3 different batches, one of which is at least 60 days old against *Staphylococcus aureus* (ATCC 6538) and either *Klebsiella pneumoniae* (aberrant, ATCC 4352) or *Enterobacter aerogenes* (ATCC 13048 or 15038). Results must show a bacterial reduction of at least 99.9 percent over the parallel control within 5 minutes. The starting inoculum of the challenge microorganisms (for initial and subsequent challenges) must be of sufficient concentration to provide at least 10^4 survivors on the parallel control surface.

Virucidal:

The effectiveness of virucides against specific viruses must be supported by efficacy data that simulates, to the extent possible in the laboratory, the conditions under which the product is intended to be used. Carrier methods that are modifications of either the AOAC Use-Dilution Method (for liquid disinfectants) or the AOAC Germicidal Spray Products as Disinfectants Method (for spray disinfectants) must be used. To simulate in-use conditions, the specific virus to be treated must be inoculated onto hard surfaces, allowed to dry, and then treated with the product according to the directions for use on the product label. One surface for each of 2 different product lots of disinfectant must be tested against a recoverable virus titer of at least 10^4 from the test surface for a specified exposure period at room temperature. Then, the virus must be assayed by an appropriate virological technique, using a minimum of four determinations per each dilution assayed. Separate studies are required for each virus. The calculated viral titers must be reported with the test results. For the data to be considered acceptable, results must demonstrate complete inactivation of the virus at all dilutions. When cytotoxicity is evident, at least a 3-log reduction in titer must be demonstrated beyond the

cytotoxic level. If the product is intended to be represented as a one-step (ready to use) virucide, an appropriate organic soil (i.e.- 5 percent blood serum) should be included with the viral inoculum.

IV. COMMENTS ON THE SUBMITTED EFFICACY STUDIES:

1. MRID 490899-08: "Pre-Saturated Towelettes for Hard Surface Disinfection", Test Organisms: *Pseudomonas aeruginosa* (ATCC 15442), *Salmonella enterica* (ATCC 10708), *and Staphylococcus aureus* (ATCC 6538) for product Hitman Wipes, by Joshua Luedtke. Study conducted at ATS Laboratories. Study completion date – July 20, 2012. ATS Project Number A12904.

The study was conducted against *Pseudomonas aeruginosa* (ATCC 15442), *Salmonella enterica* (ATCC 10708), and *Staphylococcus aureus* (ATCC 6538). Three lots (Lot No. P11130-07, Lot No. P11130-08, and Lot No. P11130-09) of the product, Hitman Wipes, were tested using the provided ATS Laboratories Protocol No. SRC52022012.CUST.1 marked as proprietary information. One lot, Lot No. P11130-9, was ≥60 days aged. The product was received as ready-to-use towelettes. The concentration of the active ingredients was determined by Case laboratories, Inc. It was demonstrated that Lot No. P11130-07 contained an average of 0.70% hydrogen peroxide and 1.13% quaternary amine, Lot No. P11130-08 contained an average of 0.70% hydrogen peroxide and 1.13% quaternary amine, and Lot No. P11130-09 contained an average of 0.70% hydrogen peroxide and 1.11% quaternary amine. A culture of the challenge microorganism was prepared from the initial broth suspension by more than three but less than thirty daily consecutive transfers of 1 loopful (10 µL) of culture in 10 mL of the appropriate growth medium for each microorganism and incubated at 35 – 37°C. *Pseudomonas aeruginosa* was cultured in Nutrient Broth. *Salmonella enterica* and *Staphylococcus aureus* was cultured in Synthetic Broth. The last culture transfer suspension was incubated 48 - 54 hours at 35 – 37°C, mixed, and allowed to stand for ≥ 10 minutes prior to removing the upper portion of the culture for use in testing. For testing performed 3/16/12, the *Pseudomonas aeruginosa* pellicle was carefully vacuum aspirated by tilting each tube to allow any remaining pellicle to slide back and away from the aspirated culture. Fetal Bovine Serum was added to the cultures to achieve a final 5% organic soil load. Glass slide carriers (3 in. x 1 in.) were inoculated with 10.0 µL of test organism suspension. The inoculum was uniformly spread over an area of 1 in. X 1 in. for each carrier and the carriers were dried for 30 minutes at 35 - 37°C and 50-51% relative humidity. After drying, one saturated towelette was used to wipe the contaminated portion of 10 carriers. The area of the towelette used was rotated so as to expose a maximum amount of the towelette surface during the course of the wiping procedure. Each inoculated carrier was treated with the towelette by passing over the carrier surface back and forth twice for a total of 4 passes. For testing performed 3/26/12, the treated carriers were held for a 5 minute exposure time at 20°C and 29% relative humidity; for testing performed 5/16/12, the treated carriers were held for a 5 minute exposure time at 20°C and 32% relative humidity; and for testing performed 6/5/12, the treated carriers were held for a 5 minute exposure time at 21°C and 55% relative humidity. Following exposure, each carrier was transferred to 40 mL of Letheen Broth + 0.14% Lecithin + 1.0% Tween 80 + 0.1% Sodium Lauryl Sulfate + 0.01% Catalase neutralizing solution. For *Staphylococcus aureus,* the carriers were transferred from the primary subcultures into individual secondary subcultures containing 40 mL of Letheen Broth + 0.07% Lecithin + 0.5% Tween 80 for ≥30 minutes following the first transfer. All subcultures were incubated for 48 ± 4 hours at 35 - 37°C. Following incubation, the subcultures were visually examined for the presence or absence of growth. Controls included those for carrier population, purity, sterility, viability, and neutralization confirmation.

Note: Two carriers in *Staphylococcus aureus* secondary subcultures showed growth with Lot P11130-07, therefore this test was repeated. Lot P11130-09 was also retested against *Staphylococcus aureus* (ATCC 6538) due to a primary neutralization jar being dropped and broke which left 59 out of 60 carriers to examine. However, the included invalid attached data shows 59 carriers for the primary subculture and 60 carriers for the secondary subculture. If one carrier was lost prior to the primary subculture then one would expect to have it also lost for the secondary subculture. Lot P11130-09 was only tested against *Staphylococcus aureus* (ATCC 6538) and *Salmonella enterica* (ATCC 10708).

2. MRID 490899-09: "Pre-Saturated Towelettes for Hard Surface Disinfection", Test Organisms: *Pseudomonas aeruginosa* (ATCC 15442), *and Staphylococcus aureus* (ATCC 6538) for Product Hitman, by Joshua Luedtke. Study conducted at ATS Laboratories. Study completion date – August 30, 2012. ATS Project Number A13108.

The study was conducted against *Pseudomonas aeruginosa* (ATCC 15442) and *Staphylococcus aureus* (ATCC 6538). Three lots (Lot No. P11130-07, Lot No. P11130-08, and Lot No. P11130-09) of the product, Hitman Wipes, were tested using the provided ATS Laboratories Protocol No. SRC52041712.CUST marked as proprietary information. One lot, Lot No. P11130-9, was ≥60 days aged. The product was received as ready-to-use towelettes. The concentration of the active ingredients was determined by Case laboratories, Inc. It was demonstrated that Lot No. P11130-07 contained an average of 0.68% hydrogen peroxide and 1.08% quaternary amine, Lot No. P11130-08 contained an average of 0.70% hydrogen peroxide and 1.13% quaternary amine, and Lot No. P11130-09 contained an average of 0.68% hydrogen peroxide and 1.04% quaternary amine. A culture of the challenge microorganism was prepared from the initial broth suspension by more than three but less than thirty daily consecutive transfers of 1 loopful (10 µL) of culture in 10 mL of the appropriate growth medium for each microorganism and incubated at 35 – 37°C. *Pseudomonas aeruginosa* was cultured in Nutrient Broth and *Staphylococcus aureus* was cultured in Synthetic Broth. The last culture transfer suspension was incubated 48 - 54 hours at 35 – 37°C, mixed, and allowed to stand for ≥ 10 minutes prior to removing the upper portion of the culture for use in testing. The *Pseudomonas aeruginosa* pellicle was carefully vacuum aspirated by tilting each tube to allow any remaining pellicle to slide back and away from the aspirated culture. The culture was mixed with a 0.20 mL aliquot of fetal bovine serum (FBS) to achieve a 5% organic soil load for tests conducted on 4/25/12. *Staphylococcus aureus* (ATCC 6538) was retested using a 5% organic soil load using FBS on 7/30/12 and no organic soil load on 8/8/12. Glass slide carriers (3 in. x 1 in.) were inoculated with 10.0 µL of test organism suspension. The inoculum was uniformly spread over an area of 1 in. X 1 in. for each carrier and on 4/25/12 the carriers were dried for 30 minutes at 35 -37°C with 52 - 53% relative humidity; on 7/30/12 the carriers were dried for 30 minutes at 35 -37°C with 50% relative humidity; and on 8/8/12 the carriers were dried for 30 minutes at 35 - 37°C with 40 - 42% relative humidity. After drying, one saturated towelette was used to wipe the contaminated portion of 10 carriers. The area of the towelette used was rotated so as to expose a maximum amount of the towelette surface during the course of the wiping procedure. Each inoculated carrier was treated with the towelette by passing over the carrier surface back and forth twice for a total of 4 passes. For testing performed 4/25/12, the treated carriers were held for a 6 minute exposure time at 21°C and 44% relative humidity; for testing performed 7/30/12, the treated carriers were held for a 6 minute exposure time at 21°C and 64% relative humidity; and for testing performed 8/8/12, the treated carriers were held for a 6 minute exposure time at 23°C and 54% relative humidity. Following exposure on 4/25/12, each *Staphylococcus aureus* (ATCC 6538) carrier was transferred to 40 mL of neutralizer (Letheen Broth + 0.14% Lecithin + 1.0% Tween 80 + 0.5% Sodium Lauryl Sulfate + 0.01% Catalase.); each *Pseudomonas*

aeruginosa (ATCC 15442) carrier was transferred to 40 mL of neutralizer (Letheen Broth + 0.14% Lecithin + 1.0% Tween 80 + 0.1% Sodium Lauryl Sulfate + 0.01% Catalase). For testing performed 7/30/12 and 8/8/12, each exposed carrier was transferred to 40 mL of D/E + 0.25% Sodium Lauryl Sulfate + 0.01% Catalase + 2.5% FBS. All subcultures were incubated for 48 ± 4 hours at 35 - 37°C. Following incubation, the subcultures were visually examined for the presence or absence of growth. All subcultures of the D/E broth for testing performed on 7/30/12/ and 8/8/12 were streaked following incubation for confirmation of test organism growth. Controls included those for carrier population, purity, sterility, viability, and neutralization confirmation.

Note: Protocol amendments were reviewed and found to be acceptable.

3. MRID 490899-10: "Pre-Saturated Towelettes for Hard Surface Disinfection", Test Organisms: *Escherichia coli O157:H7* (ATCC 35150) for product Hitman Wipes, by Joshua Luedtke. Study conducted at ATS Laboratories. Study completion date – June 19, 2012. ATS Project Number A13155.

The study was conducted against *Escherichia coli O157:H7* (ATCC 35150). Two lots, Lot No. P11130-07 and Lot No. P11130-08 (both ≥ 60 days aged at the time of testing), of the product Hitman Wipes were tested using the provided ATS Laboratories Protocol No. SRC52022012.CUST.3 marked as proprietary information. The product was received as ready-to-use towelettes. The concentration of the active ingredients was determined by Case laboratories, Inc. It was demonstrated that Lot No. P11130-07 contained an average of 0.70% hydrogen peroxide and 1.13% quaternary amine, and Lot No. P11130-08 contained an average of 0.70% hydrogen peroxide and 1.13% quaternary amine. Broth cultures of the test organisms were prepared by inoculation of an initial tube of broth from a stock slant and performing a minimum of three but less than thirty daily subculture transfers using 1 loopful (10 µL) of culture in 10 mL of Nutrient broth media. The last culture transfer suspension was incubated 48 - 54 hours at 35 – 37°C, mixed, and allowed to stand for ≥ 10 minutes prior to removing the upper portion of the culture for use in testing. Fetal Bovine Serum was added to achieve a 5% of organic soil load. Glass slide carriers (3 in. X 1 in.) were inoculated with 10.0 µL of the test organism suspension. The inoculum was uniformly spread over an approximate 1" x 1" area of each carrier and the carriers were dried for 30 minutes at 35 - 37°C with 51% relative humidity. After drying, one saturated towelette was used to wipe the contaminated portion of 10 carriers. The area of the towelette used was rotated so as to expose a maximum amount of the towelette surface during the course of the wiping procedure. Each inoculated carrier was treated with the towelette by passing it over the carrier surface back and forth twice for a total of 4 passes. The treated carriers were held for a 5 minute exposure time at 21°C and 53% relative humidity. Following exposure, each carrier was transferred to 40 mL of neutralizer (Letheen Broth + 0.14% Lecithin + 1.0% Tween 80 + 0.1% Sodium Lauryl Sulfate + 0.01% Catalase.). All subcultures were incubated for 48 ± 4 hours at 35 - 37°C. Following incubation, the subcultures were visually examined for the presence or absence of growth. Controls included those for carrier population, purity, sterility, viability, and neutralization confirmation.

Note: Protocol amendments were reviewed and found to be acceptable.

4. MRID 490899-11: "Pre-Saturated Towelettes for Hard Surface Disinfection", Test Organisms: *Klebsiella pneumoniae* (ATCC 4352) for product Hitman Wipes, by Joshua Luedtke. Study conducted at ATS Laboratories. Study completion date – June 19, 2012. ATS Project Number A13156.

The study was conducted against *Klebsiella pneumoniae* (ATCC 4352). Two lots, Lot No. P11130-07 and Lot No. P11130-08 (both ≥ 60 days aged), of the product Hitman Wipes were tested using the provided ATS Laboratories Protocol No. SRC52022012.CUST.4 marked as proprietary information. The product was received as ready-to-use towelettes. The concentration of the active ingredients was determined by Case laboratories, Inc. It was demonstrated that Lot No. P11130-07 contained an average of 0.70% hydrogen peroxide and 1.13% quaternary amine, and Lot No. P11130-08 contained an average of 0.70% hydrogen peroxide and 1.13% quaternary amine. Broth cultures of the test organisms were prepared by inoculation of an initial tube of broth from a stock slant and performing a minimum of three but less than thirty daily subculture transfers using 1 loopful (10 µL) of culture in 10 mL of Nutrient broth media. The last culture transfer suspension was incubated 48 - 54 hours at 35 – 37°C, mixed, and allowed to stand for ≥ 10 minutes prior to removing the upper portion of the culture for use in testing. Fetal Bovine Serum was added to achieve a 5% of organic soil load. Glass slide carriers (3 in. X 1 in.) were inoculated with 10.0 µL of the test organism suspension. The inoculum was uniformly spread over an approximate 1" x 1" area of each carrier and the carriers were dried for 30 minutes at 35 - 37°C and 40% relative humidity. After drying, one saturated towelette was used to wipe the contaminated portion of 10 carriers. The area of the towelette used was rotated so as to expose a maximum amount of the towelette surface during the course of the wiping procedure. Each inoculated carrier was treated with the towelette by passing it over the carrier surface back and forth twice for a total of 4 passes. The treated carriers were held for a 5 minute exposure time at 21°C with 64% RH. Following exposure, each carrier was transferred to 40 mL of neutralizer (Letheen Broth + 0.14% Lecithin + 1.0% Tween 80 + 0.1% Sodium Lauryl Sulfate + 0.01% Catalase.). All subcultures were incubated for 48 ± 4 hours at 35 - 37°C in 6.0% CO_2. Following incubation, the subcultures were visually examined for the presence or absence of growth. Controls included those for carrier population, purity, sterility, viability, and neutralization confirmation.

Note: Protocol amendments were reviewed and found to be acceptable.

5. MRID 490899-12: "Pre-Saturated Towelettes for Hard Surface Disinfection", Test Organisms: *Streptococcus pyogenes* (*ATCC 19615*) for product Hitman Wipes, by Joshua Luedtke. Study conducted at ATS Laboratories. Study completion date – June 19, 2012. ATS Project Number A13158.

The study was conducted against *Streptococcus pyogenes* (ATCC 19615). Two lots, Lot No. P11130-07 and Lot No. P11130-08 (both ≥ 60 days old), of the product Hitman Wipes were tested using the provided ATS Laboratories Protocol No. SRC52022012.CUST.6 marked as proprietary information. The product was received as ready-to-use towelettes. The concentration of the active ingredients was determined by Case laboratories, Inc. It was demonstrated that Lot No. P11130-07 contained an average of 0.68% hydrogen peroxide and 1.08% quaternary amine and Lot No. P11130-08 contained an average of 0.70% hydrogen peroxide and 1.13% quaternary amine. Cultures of the test organisms were prepared by inoculation of multiple agar plates and incubating for 4 days at 35 - 37°C in 6.0% CO_2. Following incubation, an organism suspension was prepared in Fluid Thioglycollate Medium to target 1 x 10^8 CFU/mL. Fetal bovine serum was added to achieve a 5% organic soil load. Glass slide carriers (3 in. X 1 in.) were inoculated with 10.0 µL of test organism suspension. The inoculum was uniformly spread over an approximate 1" x 1" area of each carrier and the carriers were dried for 30 minutes at 25 - 30°C with 65% relative humidity. After drying, one saturated towelette was used to wipe the contaminated portion of 10 carriers. The area of the towelette used was rotated so as to expose a maximum amount of the towelette surface during

the course of the wiping procedure. Each inoculated carrier was treated with the towelette by passing over the carrier surface back and forth twice for a total of 4 passes. The treated carriers were held for a 5 minute exposure time at 21°C with 53% relative humidity. Following exposure, each carrier was transferred to 40 mL of neutralizer (Letheen Broth + 0.14% Lecithin + 1.0% Tween 80 + 0.1% Sodium Lauryl Sulfate + 0.01% Catalase.). The carriers were then transferred from the primary subcultures into individual secondary subcultures containing 40 mL of Brain Heart Infusion Broth ≥ 30 minutes following the first transfer. All subcultures were incubated for 48 ± 4 hours at 35 - 37°C in 6.0% CO_2. Following incubation, the subcultures were visually examined for the presence or absence of growth. Controls included those for carrier population, purity, sterility, viability, and neutralization confirmation.

Note: Protocol amendments were reviewed and found to be acceptable.

6. MRID 490899-13 "Virucidal Efficacy of Pre-Saturated Towelettes for Hard Surface Disinfection," Virus: Influenza A (H1N1) virus, for product Hitman Wipe, by Mary J. Miller, M.T. Study conducted at ATS Labs. Study completion date – June 4, 2012. Project Number A13067.

The study was conducted against Influenza A (H1N1) virus, ATCC VR-1469, Strain A/PR/8/34. Three lots of test substance Hitman Wipe, Lot P11130-07, Lot P11130-08 and Lot P11130-09 (all lots >60 days old) were tested using the provided ATS Laboratory Protocol No. SRC52030512.FLUA marked as proprietary information. The host cell line was Rhesus Monkey Kidney (RMK) cells, obtained from ViroMed Laboratories Cell Culture Division. The concentration of the active ingredients was determined by Case laboratories, Inc. It was demonstrated that Lot No. P11130-07 contained an average of 0.70% hydrogen peroxide and 1.13% quaternary amine, Lot No. P11130-08 contained an average of 0.70% hydrogen peroxide and 1.13% quaternary amine, and Lot No. P11130-09 contained an average of 0.70% hydrogen peroxide and 1.11% quaternary amine. The stock virus was prepared by collecting the supernatant culture fluid from 75 – 100% infected culture cells that were disrupted and cell debris removed by centrifugation. The high titer stock virus was aliquoted and stored at ≤-70°C until the day of use then three aliquots of stock virus (ATS Lot FLUA-34) were removed, thawed, and combined. The stock virus culture contains a final soil load of 5% fetal bovine serum. Films of virus were prepared by spreading 200 µL of virus uniformly over approximately 8 x 8 cm, on the bottoms of twelve 150 x 15 mm sterile glass Petri dishes and dried at 20.0°C for 20 minutes at 40% relative humidity. The test substance Hitman Wipe consisting of single use towelettes impregnated with the active ingredient, were folded widthwise two times and lengthwise two times to form a 2 x 2 inch square for use in testing and were pre-equilibrated to the exposure temperature prior to use. For each lot of test substance, using sterile gloves, the dried virus film on the surface of three inoculated glass Petri dishes was divided into two sections and each section was wiped with a separate, saturated towelette over and back two times for a total of four passes. The area of towelette used for wiping was rotated so as to expose a maximum amount of its surface. The Petri dishes were covered and held at 20.0°C in a relative humidity of 40% for 5 minutes. Following the exposure time, a 2.00 mL aliquot of test medium was added to each Petri dish, and the plates were individually scraped with a cell scraper to re-suspend the contents (10^{-1} dilution) and the virus-test substance mixtures were immediately passed through prepared individual Sephadex columns utilizing the syringe plungers to detoxify the mixtures. The filtrates were then passed through a second Sephadex column to aid in removing the cytotoxic effect of the test substance to the indicator cell cultures. Each filtrate (10^{-1} dilution) was immediately titered by 10-fold serial dilution and was then assayed for infectivity and/or cytotoxicity. The RMK cells in multiwell culture dishes were inoculated in quadruplicate with 100 µL of the dilutions from the test and control groups and were incubated at 36 - 38°C in a

humidified atmosphere of 5 - 7% CO_2. The cultures were microscopically scored periodically for 7 days for the absence or presence of cytopathy, cytotoxicity, and for viability. Controls included those for dried virus film recovery, cytotoxicity, neutralization and cell viability. Viral and cytotoxicity titers were calculated by the method of Spearman Karber.

Note: Protocol deviations/amendments reported in this study were reviewed and found to be acceptable.

7. MRID 490899-14 "Virucidal Efficacy of Pre-Saturated Towelettes for Hard Surface Disinfection," Virus: Respiratory syncytial virus (RSV), for product Hitman Wipe, by Mary J. Miller, M.T. Study conducted at ATS Labs. Study completion date – June 11, 2012. Project Number A13066.

The study was conducted against Respiratory syncytial virus (RSV), ATCC VR-26, Strain Long. Three lots of test substance Hitman Wipe, Lot P11130-07, Lot P11130-08 and Lot P11130-09 (all lots >60 days old) were tested using the provided ATS Laboratory Protocol No. SRC52030512.RSV marked as proprietary information. The host cell line, Hep-2 (human larynx carcinoma) cells, obtained from ViroMed Labs Cell Culture Division, were maintained and used in tissue culture at 36 - 38°C in a humidified atmosphere at 5 - 7% CO_2. The concentration of the active ingredients was determined by Case laboratories, Inc. It was demonstrated that Lot No. P11130-07 contained an average of 0.70% hydrogen peroxide and 1.13% quaternary amine, Lot No. P11130-08 contained an average of 0.70% hydrogen peroxide and 1.13% quaternary amine, and Lot No. P11130-09 contained an average of 0.70% hydrogen peroxide and 1.11% quaternary amine. The stock virus was prepared by collecting the supernatant culture fluid from 75 – 100% infected culture cells that were disrupted and cell debris removed by centrifugation. The high titer stock virus was aliquoted and stored at ≤-70°C until the day of use then three aliquots of stock virus (ATS Lot NRSV-28) were removed, thawed, and combined. The stock virus culture contains a final soil load of 5% fetal bovine serum. Films of virus were prepared by spreading 200 µL of virus uniformly over approximately 8 x 8 cm, on the bottoms of 12 separate 150 x 15 mm sterile glass Petri dishes and dried at 20.0°C for 20 minutes at 50% relative humidity. The test substance Hitman Wipe consisting of single use towelettes impregnated with the active ingredient, were folded widthwise two times and lengthwise two times to form a 2 x 2 inch square for use in testing and were pre-equilibrated to the exposure temperature prior to use. For each lot of test substance, using sterile gloves, the dried virus film on the surface of three inoculated glass Petri dishes was divided into two sections and each section was wiped with a separate, saturated towelette over and back two times for a total of four passes. The area of towelette used for wiping was rotated so as to expose a maximum amount of its surface. The Petri dishes were covered and held at 20.0°C in a relative humidity of 50% for 5 minutes. Following the exposure time, a 2.00 mL aliquot of test medium was added to each Petri dish, and the plates were individually scraped with a cell scraper to re-suspend the contents (10^{-1} dilution) and the virus-test substance mixtures were immediately passed through prepared individual Sephadex columns utilizing the syringe plungers to detoxify the mixtures. The filtrates were then passed through a second Sephadex column to aid in removing the cytotoxic effect of the test substance to the indicator cell cultures. Each filtrate (10^{-1} dilution) was immediately titered by 10-fold serial dilution and was then assayed for infectivity and/or cytotoxicity. The Hep-2 cells in multiwell culture dishes were inoculated in quadruplicate with 100 µL of the dilutions from the test and control groups and were incubated at 36 - 38°C in a humidified atmosphere of 5 - 7% CO_2. The cultures were scored periodically for 10 days for the absence or presence of CPE, cytotoxicity, and for viability. Controls included those for dried virus film recovery, cytotoxicity, neutralization and cell viability. Viral and cytotoxicity titers were calculated by the method of Spearman Karber.

Note: Protocol deviations/amendments reported in this study were reviewed and found to be acceptable. The assay was originally conducted on April 20, 2013 and was repeated on May 11, 2012, to recover at least 4 logs of infectivity from the dried virus control replicates beyond the cytotoxic level of the test substance. The Input Virus Control for the invalid data developed on April 20, 2012 average $TCID_{50}$/100 µL was $10^{4.50}$ Log and cytotoxicity was seen at 10^{-1} for all tested lots.

8. MRID 490899-15 "Virucidal Efficacy of Pre-Saturated Towelettes for Hard Surface Disinfection," Virus: Human Coronavirus, for product Hitman Wipe, by Mary J. Miller, M.T. Study conducted at ATS Labs. Study completion date – June 1, 2012. Project Number A13065.

The study was conducted against Human Coronavirus, ATCC VR-740, Strain 229E. Three lots of test substance Hitman Wipe, Lot P11130-07, Lot P11130-08 and Lot P11130-09 (all lots >60 days aged), were tested using the provided ATS Laboratory Protocol No. SRC52030512.COR marked as proprietary information. The host cell line, WI-38 (human lung) cells, ATCC CCL-75, were maintained and used in tissue culture at 36 - 38°C in a humidified atmosphere at 5 - 7% CO_2. The concentration of the active ingredients was determined by Case laboratories, Inc. It was demonstrated that Lot No. P11130-07 contained an average of 0.70% hydrogen peroxide and 1.13% quaternary amine, Lot No. P11130-08 contained an average of 0.70% hydrogen peroxide and 1.13% quaternary amine, and Lot No. P11130-09 contained an average of 0.70% hydrogen peroxide and 1.11% quaternary amine. The stock virus was prepared by collecting the supernatant culture fluid from 75 – 100% infected culture cells that were disrupted and cell debris removed by centrifugation. The high titer stock virus was aliquoted and stored at ≤-70°C until the day of use then three aliquots of stock virus (ATS Lot HCV-66) were removed, thawed, and combined. The stock virus culture contains a final soil load of 5% fetal bovine serum. Films of virus were prepared by spreading 200 µL of virus uniformly over approximately 8 x 8 cm, on the bottoms of 12 separate 100 x 15 mm sterile glass Petri dishes and dried at 20.0°C for 20 minutes at 50% relative humidity. The test substance Hitman Wipe consisting of single use towelettes impregnated with the active ingredient, were folded widthwise two times and lengthwise two times to form a 2 x 2 inch square for use in testing and were pre-equilibrated to the exposure temperature prior to use. For each lot of test substance, using sterile gloves, the dried virus film on the surface of three inoculated glass Petri dishes was divided into two sections and each section was wiped with a separate, saturated towelette over and back two times for a total of four passes. The area of towelette used for wiping was rotated so as to expose a maximum amount of its surface. The Petri dishes were covered and held at 20.0°C in a relative humidity of 50% for 5 minutes. Following the exposure time, a 2.00 mL aliquot of test medium was added to each Petri dish, and the plates were individually scraped with a cell scraper to re-suspend the contents (10^{-1} dilution) and the virus-test substance mixtures were immediately passed through prepared individual Sephadex columns utilizing the syringe plungers to detoxify the mixtures. The filtrates were then passed through a second Sephadex column to aid in removing the cytotoxic effect of the test substance to the indicator cell cultures. Each filtrate (10^{-1} dilution) was immediately titered by 10-fold serial dilution and was then assayed for infectivity and/or cytotoxicity. The WI-38 cells in multiwell culture dishes were inoculated in quadruplicate with 100 µL of the dilutions from the test and control groups and were incubated at 36-38°C in a humidified atmosphere of 5-7% CO_2. The cultures were scored periodically for 10 days for the absence or presence of CPE, cytotoxicity, and for viability. Controls included those for dried virus film recovery, cytotoxicity, neutralization and cell viability. Viral and cytotoxicity titers were calculated by the method of Spearman Karber.

Note: No protocol deviations or amendments were required for this study. The assay was

originally conducted on April 19, 2012 and was repeated on May 10, 2012, to recover at least 4 logs of infectivity from the dried virus control replicates beyond the cytotoxic level of the test substance for each of the three lots. In the invalid data, cytotoxicity was seen at 10^{-1} to 10^{-2} for each lot.

9. MRID 490899-16 "EPA Hard Surface Mildew-Fungistatic Test." Test Organism: *Aspergillus niger* (ATCC 6275), for product Hitman Wipe, by Joshua Luedtke, M.S. Study conducted at ATS Labs. Study completion date – June 21, 2012. Project Number A13017.

The study was conducted against *Aspergillus niger* (ATCC 6275). Two lots of test substance Hitman Wipe, Lot No. P11130-07 and Lot No. P11130-08, were tested using the provided ATS Laboratory Protocol No. SRC52022012.MSTAT marked as proprietary information. The concentration of the active ingredients was determined by Case laboratories, Inc. It was demonstrated that Lot No. P11130-07 contained an average of 0.70% hydrogen peroxide and 1.13% quaternary amine and Lot No. P11130-08 contained an average of 0.70% hydrogen peroxide and 1.13% quaternary amine. The stock *Aspergillus niger* conidial suspension was prepared by inoculating a flask of Sabouraud Agar with the stock culture and incubating for 10 days at 25 - 30°C. When the cultures were mature, the mycelia mats were removed from the surface and filtered through sterile gauze to remove the hyphal fragments, then the suspension was macerated in a tissue grinder to break up spore chains. The final conidial suspension was standardized to contain approximately 1 x 10^7 conidia/mL by combining 1.00 mL of culture with 9.0 mL of sterile 0.85% saline + 0.05% Triton X-100. A 1.00 mL aliquot was added to 20.0 mL of sterile Czapek's solution. Fetal bovine serum was added to achieve a 5% organic soil load. For each lot of test substance, 10 carriers were treated with a towelette saturated with the test substance, by wiping the glazed area with the towelette over and back two times for a total of 4 passes. The treated carriers were placed in a near vertical position to permit excess liquid to drain and were then dried in Petri dishes at 35 - 37°C for 35 minutes. Following the drying period, an atomizer was used to spray 5 sprays onto the surface of each test and control carrier with the *Aspergillus niger* conidia-Czapek suspension. They were returned to a 35 - 37°C incubator and dried for 45 minutes until dry. Each carrier (sprayed side up) was placed onto an individual water agar plate and incubated at 25-30°C in a minimum of 95% humidity. All test and control carriers were examined after 7 days for visible growth. Controls included purity, carrier sterility, agar sterility, and organic soil load sterility.

Note: No protocol deviations or amendments were required for this study.

10. MRID 490899-17 "Efficacy of Sanitizers Recommended for Inanimate Non-Food Contact Surfaces" Test Organisms: *Enterobacter aerogenes* (ATCC 13048) and *Staphylococcus aureus* (ATCC 6538) for product Hitman Wipes, by Jessica Akins. Study conducted at Antimicrobial Test Laboratories. Study completion date – June 27, 2012. Project Number GLP1106.

The study was conducted against *Enterobacter aerogenes* (ATCC 13048) *Staphylococcus aureus* (ATCC 6538). Three lots, Lot No. P11130-07, Lot No. P11130-08, and Lot No. P11130-09 (≥ 60 days old), of the product Hitman Wipes were tested using the provided Antimicrobial Test Laboratories Protocol No. P1118. The product was received as ready-to-use towelettes. The concentration of the active ingredients was determined by Case laboratories, Inc. It was demonstrated that Lot No. P11130-07 contained an average of 0.70% hydrogen peroxide and 1.13% quaternary amine, Lot No. P11130-08 contained an average of 0.70% hydrogen peroxide and 1.13% quaternary amine, and Lot No. P11130-09 contained an average

of 0.70% hydrogen peroxide and 1.11% quaternary amine. The challenge microorganisms were prepared by initiating a daily culture from the monthly working stock culture, which has a transfer number ≤ 5, for each microorganism and a loopful transferred once daily in nutrient 10 mL broth, at least three times consecutively prior to initiation of the test culture. The final subculture was incubated for 48 - 54 hours at 35 - 37°C for *S. aureus* and 29 - 31 °C for *E. aerogenes*. Fetal Bovine Serum was added to achieve a 5.0% organic soil load. Five (5) 3 x 1 inch sterile glass slide carriers per product lot were inoculated with 20.0 µL of test organism suspension. The inoculum was uniformly spread over approximately 1 sq. in. of each carrier staying within 3mm of the edge of the carrier. The carriers were dried for 36 - 37 minutes at 35 - 37°C with 38 - 41% relative humidity for *E. aerogenes and* 35-40% for *S. aureus*. After drying, each of the five test carriers was wiped with a single saturated towelette. The area of the towelette used was rotated so as to expose a maximum amount of the towelette surface during the course of the wiping procedure. Each inoculated carrier was treated with the towelette by passing over the carrier surface back and forth twice for a total of 4 passes. The carriers were allowed to expose for 15 seconds. Following exposure, each carrier was transferred to 20 mL of neutralizer (2X concentrated Dey/Engley broth + 0.25% sodium lauryl sulfate + 0.1% catalase) and eluted using a cell scraper. Within 30 minutes of neutralization, the eluted carriers are plated in duplicate using 0.9 mL centrifuge tubes containing PBS using standard plating technique. Controls included those for carrier population, purity, sterility, viability, and neutralization confirmation.

Note: Protocol deviations were reviewed and found to be acceptable.

11. MRID 490899-18 "Efficacy of Sanitizers Recommended for Inanimate Non-Food Contact Surfaces" Test Organism: Community Acquired Methicillin Resistant *Staphylococcus aureus* CA-MRSA (NARSA NRS123) (Genotype USA400) for product Hitman Wipes, by Jessica Akins. Study conducted at Antimicrobial Test Laboratories. Study completion date – June 27, 2012. Project Number GLP1108.

The study was conducted against *Staphylococcus aureus* CA-MRSA (NARSA NRS123) (Genotype USA400). Two lots, Lot No. P11130-07 and Lot No. P11130-08, of the product Hitman Wipes were tested using the provided Antimicrobial Test Laboratories Protocol No. P1119. The product was received as ready-to-use towelettes. The concentration of the active ingredients was determined by Case Laboratories, Inc. It was demonstrated that Lot No. P11130-07 contained an average of 0.70% hydrogen peroxide and 1.13% quaternary amine and Lot No. P11130-08 contained an average of 0.70% hydrogen peroxide and 1.13% quaternary amine. The challenge microorganisms were prepared by initiating a daily culture from the monthly working stock culture, which has a transfer number ≤ 5, and a loopful transferred once daily in 10 mL of synthetic broth, at least three times consecutively prior to initiation of the test culture. The final subculture was incubated for 48 - 54 hours. Fetal Bovine Serum was added to achieve a 5.0% organic soil load. Five (5) 3 x 1 inch sterile glass slide carriers per product lot were inoculated with 20.0 µL of test organism suspension. The inoculum was uniformly spread over approximately 1 sq. in. of each carrier staying within 3 mm of the edge of the carrier. The carriers were dried for 35 minutes at 36 ± 1°C with 38 - 40% relative humidity. After drying, each of the five test carriers was wiped with a single saturated towelette. The area of the towelette used was rotated so as to expose a maximum amount of the towelette surface during the course of the wiping procedure. Each inoculated carrier was treated with the towelette by passing over the carrier surface back and forth twice for a total of 4 passes. The carriers were allowed to expose for 15 seconds. Following exposure, each carrier was transferred to 20 mL of neutralizer (2X concentrated Dey/Engley broth + 0.25% sodium lauryl

sulfate + 0.1% catalase) and eluted using a cell scraper. Within 30 minutes of neutralization, the eluted carriers are plated in duplicate using 0.9 mL centrifuge tubes containing PBS using standard plating technique. The plates were incubated at 36 ± 1°C for 48 hours and 26 minutes. Controls included those for carrier population, purity, sterility, viability, neutralization confirmation. Antibiotic resistance was confirmed using Mueller Hinton agar plate and 1µg Oxacillin disk.

Note: Protocol amendments were reviewed and found to be acceptable. Verification of antibiotic resistance was performed using the Mueller Hinton agar plate and 1µg Oxacillin disk. The zone of inhibition ≤10 millimeters around the disk is considered resistant and was demonstrated for the test organism *S. aureus* CA-MRSA (NRS 123).

12. MRID 490889-19 "Residual Self-Sanitizing Activity of Dried Chemical Residues on Hard Nonporous Surfaces, with Exposure and Wear Activity." Test organisms, *Enterobacter aerogenes* (ATCC 13048) and *Staphylococcus aureus* (ATCC 6538)," for product Hitman Wipe, by Laura Higgins. B.S. Performing Laboratory, Antimicrobial Test Laboratories. Study completion date, June 29, 2012. Study ID Number GLP1107.

The study was conducted against *Enterobacter aerogenes* (ATCC 13048) and *Staphylococcus aureus* (ATCC 6538). Three lots of test substance Hitman Wipes, Lot P11130-07, Lot P11130-08, and Lot P11130-09 (all lots > 60 days old), were tested using the provided Antimicrobial Test Laboratories Protocol No P1112. The product was received ready-to-use as towelette wipes. The concentration of the active ingredients was determined by Case laboratories, Inc. It was demonstrated that Lot No. P11130-07 contained an average of 0.70% hydrogen peroxide and 1.13% quaternary amine, Lot No. P11130-08 contained an average of 0.70% hydrogen peroxide and 1.13% quaternary amine, and Lot No. P11130-09 contained an average of 0.70% hydrogen peroxide and 1.11% quaternary amine. The challenge microorganisms were prepared by initiating a broth culture of test organism in 10 mL of nutrient broth and incubating for 18 – 24 hours. At minimal 3 daily transfers of a loopful of organism in 10 mL of nutrient broth were made. The final culture was incubated for 48 – 54 hours at 30 ± 3°C for *E. aerogenes* and 35 ± 2°C for *S. aureus*. Fetal bovine serum was added to each inoculum to achieve a 5% organic soil load. Four sterile 1" x 1" glass microscope slide carriers per product lot, per microorganism were used. Each carrier was inoculated with 0.01 mL of challenge microorganism and immediately spread to within 1/8 inch of the edge. The carriers were then dried for 32 minutes at 35 ± 2°C at 45 - 55% relative humidity. The test substance Hitman Wipe consisting of single use towelettes impregnated with the active ingredient, were folded widthwise two times and lengthwise two times to form a 2 x 2 inch square for use in testing. Four dry carriers per lot per microorganism were treated by wiping over and back two times for a total of 4 passes then were placed in separate Petri dishes with the lids ajar. Four dry control carriers per test organism were treated with 0.01% Trition X-100 solution by spraying for 3 seconds with a Preval sprayer. After treatment, all of the test and control carriers were allowed to dry overnight (15 hours and 45 minutes) at room temperature and 45 - 55% relative humidity. The test and control carriers underwent 12 wear cycles (alternating between 6 wet and 6 dry) and 5 re-inoculations with the mean CFU/mL of 1.83 X 10^3 for *E. aerogenes* and 1.11 X 10^3 for *S. aureus* which were alternated with the first five abrasions. Abrasions were conducted at room temperature and 45 - 55% relative humdity using a 1084 ± 1 gram abrasion boat on a Gardco Washability & Wear Tester set for a speed of 2.25 - 2.5 (total surface contact time of 4 - 5 seconds). Liners and clothes were replaced between each wear cycle. After a wear cycle, carriers are allowed to sit for at least 15 minutes before being re-inoculated. Carriers were re-inoculated with 0.01 mL of the re-inoculation culture with 5% soil and spread to within 1/8 inch of

the edge, then allowed to dry at ambient temperature for at least 30 minutes prior to the subsequent wear cycle. Approximately 46 - 47.5 hours after carrier test/control treatment, wear and re-inoculation cycling, the carriers were inoculated with 0.01 mL of the 18 - 24 hour old sanitization test culture. Five minutes after inoculation, carriers were transferred to sterile tube containing 30 mL of 2X concentrated D/E neutralizer broth with 0.1% catalase. The tubes were sonicated for 20 ± 2 seocnds, followed by agitation on an orbital shaker for 3 - 4 minutes at 250 rpm to suspend the surviving organisms. The neutralized solutions were serially diluted in 0.9 mL sterile R/O water. Duplicate pour plates of the 10^{-1} and 10^{-3} dilutions for the test carriers were plated on TSA. All subcultures were incubated for 48 - 54 hours at 35 ± 2°C for *S.aureus* and 30 ± 2°C for *Enterobacter aerogenes*. Following incubation, the subcultures were examined for the presence or absence of visible growth. Controls included those for purity, sterility, viability, and neutralization confirmation.

Note: Protocol deviations/amendments reported in this study were reviewed and found to be acceptable. A re-test of *E. aerogenes* Lot No. P11130-07 was performed to assess for false positives and confirm appropriate wiping technique.

13. MRID 490889-20 "Residual Self-Sanitizing Activity of Dried Chemical Residues on Hard Nonporous Surfaces, with Exposure and Wear Activity." Test organism, Community Acquired Methicillin Resistant *Staphylococcus aureus* (CA-MRSA), for product Hitman Wipe, by Benjamin Tanner, Ph.D. Performing Laboratory, Antimicrobial Test Laboratories. Study completion date, June 29, 2012. Study ID Number GLP1112.

The study was conducted against Community Acquired Methicillin Resistant *Staphylococcus aureus* -CA-MRSA (NARSA NRS123, Genotype USA 400). Two lots of test substance Hitman Wipes, Lot P11130-07 and Lot P11130-08 (both > 60 days old), were tested using the provide Antimicrobial Test Laboratories Protocol No P1123. The product was received ready-to-use as towelette wipes. The challenge microorganisms were prepared by initiating a broth culture of test organism in 10 mL of synthetic broth and incubating for 18 – 24 hours. At minimal 3 daily transfers of a loopful of organism in 10 mL of nutrient broth were made. The final culture was incubated for 48 – 54 hours at 35 ± 2°C. Fetal bovine serum was added to each inoculum to achieve a 5% organic soil load. Four sterile 1" x 1" glass microscope slide carriers per product lot, per microorganism were used. Each carrier was inoculated with 0.01 mL of challenge microorganism and immediately spread to within 1/8 inch of the edge. The carriers were then dried for 30 minutes at 35 ± 2°C at 45-55% relative humidity. The test substance Hitman Wipe consisting of single use towelettes impregnated with the active ingredient, were folded widthwise two times and lengthwise two times to form a 2 x 2 inch square for use in testing. Four dry carriers per lot per microorganism were treated by wiping over and back two times for a total of 4 passes then were placed in separate Petri dishes with the lids ajar. Four dry control carriers per test organism were treated with 0.01% Trition X-100 solution by spraying for 3 seconds with a Preval sprayer. After treatment, all of the test and control carriers were allowed to dry overnight (approx. 16 hours and 38 or 45 minutes, depending on lot) at room temperature and 45 - 55% relative humidity. The test and control carriers underwent 12 wear cycles (alternating between 6 wet and 6 dry) and 5 re-inoculations with mean CFU/mL of 3.70×10^4 which were alternated with the first five abrasions. Abrasions were conducted at room temperature and 45 - 55% relative humdity using a 1084 ± 1 gram abrasion boat on a Gardco Washability & Wear Tester set for a speed of 2.25 - 2.5 (total surface contact time of 4 - 5 seconds). Liners and clothes were replaced between each wear cycle. After a wear cycle, carriers are allowed to sit for at least 15 minutes before being re-inoculated. Carriers were re-inoculated with 0.01 mL of the re-inoculation culture with 5% soil and spread to

within 1/8 inch of the edge, then allowed to dry at ambient temperature for at least 30 minutes prior to the subsequent wear cycle. Approximately 44.5 - 45.0 hours after carrier test/control treatment, wear and re-inoculation cycling, the carriers were inoculated with 0.01 mL of the 18 - 24 hour old sanitization test culture. Five minutes after inoculation, carriers were transferred to sterile tube containing 30 mL of 2X concentrated D/E neutralizer broth with 0.1% catalase. The tubes were sonicated for 20 ± 2 seocnds, followed by agitation on an orbital shaker for 3 - 4 minutes at 250 rpm to suspend the surviving organisms. The neutralized solutions were serially diluted in 0.9 mL sterile R/O water. Duplicate pour plates of the 10^{-1} and 10^{-3} dilutions for the test carriers were plated on TSA. All subcultures were incubated for 48 - 54 hours at 35 ± 2°C. Following incubation, the subcultures were examined for the presence or absence of visible growth. Controls included those for purity, sterility, viability, and neutralization confirmation.

Note: Antimicrobial susceptibility testing was performed for Community Acquired Methicillin Resistant *Staphylococcus aureus* – CA-MRSA (NARSA NRS123, Genotype USA 400) to verify the antibiotic resistance pattern stated. The Kirby Bauer susceptibility assay was performed utilizing a representative culture from the day of testing and using oxacillin antibiotic disks to test for methicillin resistance. *Staphylococcus aureus* (ATCC 25923) was the Methicillin Resistant Quality Control (QC) organism. The zone of inhibition in mm was in the CLSI acceptable range for the test organism and the QC organism.

14. MRID 490899-21 "Kimberly Clark Final Report for Confirmation of Initial Treatment Wetness for Residual Self Sanitizing Pre-Saturated Towelette/Wipe Product," for product Hitman Wipes, by Jason Williams, B.S. Study conducted at Antimicrobial Test Laboratories. Study completion date – June 27, 2012. Study Identification Number GLP1110.

The study was conducted to document the initial surface wetness imparted by simulating use of a residual self-sanitizing towelette/wipe on a 12" x 12" test carrier. Three lots of test substance Hitman Wipe (ready to use), Lot P11130-07, Lot P11130-08, and Lot P11130-09 (all lots ≥60 days old), were tested using the provided Antimicrobial Test Laboratories Protocol No. P1111. A DVD was prepared to document the test procedure and visual wetness. The test carriers were composed of new Formica (Black Matte Finish) cut to a 12" x 12" size. The back of each carrier was labeled with a unique identifier by permanent marker. One towelette was used to wipe each 12" x 12" test carrier. Three (3) towelettes were evaluated per lot. To begin the study, the video recording of the study was initiated. The beginning temperature was 26.0°C and relative humidity of the testing area was 40.0%. Using sterile gloves the first test carrier was weighed prior to treatment using a calibrated lab balance with an accuracy of 0.01g. The test substance Hitman Wipes, consisting of single use towelettes impregnated with the active ingredient, were visually checked for uniform wetness prior to use, and then were folded widthwise two times and lengthwise two times to form a 2 x 2 inch square. A wipe was placed on the top left corner of the test carrier and was wiped in an up and down motion, each stroke slightly overlapping the last, until the entire carrier was covered (approx 5 - 7 strokes for one 12" x 12" carrier). The camera was then placed in a position to visualize the wet film on the carriers. A calibrated timer was started and the carrier was placed on the balance. The initial weight of carrier was recorded and the carrier was allowed to sit undisturbed for 15 ± 2 seconds. Upon completion of the contact time, the final wet weight of the carrier was recorded and a single sheet of unfolded cigarette paper was immediately wiped across the test surface to verify the visual wetness for the video camera. Visual wetness of the paper was defined as wetness and was observed and recorded. The ending temperature of the testing area was 26.0°C and the relative humidity was 38.0%. Wetness Determination Calculations were performed for each carrier.

V. RESULTS:

MRID Number	Organism	No. Exhibiting Growth/Total No. Tested @ 5 Minutes			Carrier Population (CFU/Carrier) (Log)
		Lot # P11130-07	Lot # P11130-08	Lot # P11130-09 (≥ 60 days old)	
490899-08	*Pseudomonas aeruginosa* (ATCC 15442)	2/60	4/60	Not tested	1.11×10^6
490899-08	*Salmonella enterica* (ATCC 10708)	0/60	0/60		1.30×10^{5a}
				0/60[b]	4.7×10^{5b}
490899-08	*Staphylococcus aureus* (ATCC 6538)	1° = 0/60[a] 2° = 2/60	1° = 1/60 2° = 0/60		2.92×10^{6a}
		1° = 0/60[c] 2° = 0/60			4.0×10^{6c}
				1° = 0/60[d] 2° = 0/60	1.13×10^{6d}
490899-10	*Escherichia coli O157:H7* (ATCC 35150)	0/10[e]	0/10[e]	Not tested	8.6×10^5
490899-11	*Klebsiella pneumonia* (ATCC 4352)	0/10[e]	0/10[e]	Not tested	1.19×10^6
490899-12	*Streptococcus pyogenes* (ATCC 19615)	1° = 0/10[e] 2° = 0/10[e]	1° = 0/10[e] 2° = 0/10[e]	Not tested	2.19×10^6

[a]Tested 3/26/12.
[b]Tested 5/16/12.
[c]Retested 5/16/12; Lot P11130-07 was ≥ 60 days old at date of retest.
[d]Tested 6/5/12.
[e]≥ 60 days aged at the time of testing.

MRID Number	Organism	No. Exhibiting Growth/Total No. Tested @ 6 Minutes			Carrier Population (CFU/Carrier) (Log)
		Lot # P11130-07	Lot # P11130-08	Lot # P11130-09 (≥ 60 days old)	
490899-09	*Pseudomonas aeruginosa* (ATCC 15442)	1/60	2/60	3/60	7.9×10^6
490899-09	*Staphylococcus aureus* (ATCC 6538)	0/60	0/60		8.4×10^6
		0/60[a]		3/60[a]	5.2×10^{6a}
				0/60[b]	3.6×10^{6b}

[a]Tested on 7/30/12 with soil organic load.
[b]Retested on 8/8/12 without soil organic load.

MRID Number	Organism		Results @ 5 Minute Contact Time $TCID_{50}$/100 µL			Dried Virus Count
			Lot # (>60 days old) P11130-07	Lot# (>60 days old) P11130-08	Lot# (>60 days old) P11130-09	
490899-13	Influenza A virus (H1N1), ATCC VR-1469	10^{-1} to 10^{-7} dilutions No cytotoxicity	Complete Inactivation	Complete Inactivation	Complete Inactivation	$10^{4.60}$
			1 $\leq 10^{0.50}$ 2 $\leq 10^{0.50}$ 3 $\leq 10^{0.50}$	1 $\leq 10^{0.50}$ 2 $\leq 10^{0.50}$ 3 $\leq 10^{0.50}$	1 $\leq 10^{0.50}$ 2 $\leq 10^{0.50}$ 3 $\leq 10^{0.50}$	
		Log_{10} Reduction	≥4.10	≥4.10	≥4.10	
490899-14	RSV ATCC VR-26, Strain Long	10^{-1} to 10^{-6} dilutions No cytotoxicity	Complete Inactivation	Complete Inactivation	Complete Inactivation	$10^{5.24}$
			1 $\leq 10^{0.50}$ 2 $\leq 10^{0.50}$ 3 $\leq 10^{0.50}$	1 $\leq 10^{0.50}$ 2 $\leq 10^{0.50}$ 3 $\leq 10^{0.50}$	1 $\leq 10^{0.50}$ 2 $\leq 10^{0.50}$ 3 $\leq 10^{0.50}$	
		Log Reduction	≥4.74	≥4.74	≥4.74	
490899-15	Human Coronavirus, ATCC VR-740	10^{-1} to 10^{-6} dilutions No cytotoxicity	Complete Inactivation	Complete Inactivation	Complete Inactivation	$10^{5.30}$
			1 $\leq 10^{0.50}$ 2 $\leq 10^{0.50}$ 3 $\leq 10^{0.50}$	1 $\leq 10^{0.50}$ 2 $\leq 10^{0.50}$ 3 $\leq 10^{0.50}$	1 $\leq 10^{0.50}$ 2 $\leq 10^{0.50}$ 3 $\leq 10^{0.50}$	
		Log Reduction	≥4.80	≥4.80	≥4.80	

MRID Number	Organism	No. Exhibiting Growth/Total No. Tested @ 7 Days		Untreated Controls
		Lot # P11130-07	Lot # P11130-08	
490899-16	*Aspergillus Niger* (ATCC 6275)	0/10	0/10	89.5% coverage Pass, where ≥50% coverage is passing.

MRID Number	Organism	Lot No.	Average No. Surviving (CFU/carrier) Log	Control Enumeration (CFU/carrier) Log	Percent Reduction
15-Second Exposure Time					
490899-17	*Enterobacter aerogenes* (ATCC 13048)	P11130-07 P11130-08 P11130-09	$<1.0 \times 10^1$ $<3.44 \times 10^1$ $<3.23 \times 10^1$	2.34×10^6 2.34×10^6 3.42×10^6	>99.9994 >99.998 >99.9989
490899-17	*Staphylococcus aureus* (ATCC 6538)	P11130-07 P11130-08 P11130-09	$<1.15 \times 10^1$ $<1.25 \times 10^1$ $<1.25 \times 10^1$	7.71×10^6 7.71×10^6 6.07×10^6	>99.9998 >99.9998 >99.9998
490899-18	*Staphylococcus aureus* CA-MRSA (NARSA NRS123) (Genotype USA400)	P11130-07 P11130-08	7.83×10^2 8.81×10^2	8.38×10^6 8.38×10^6	>99.992 >99.991

Residual Self-Sanitizing Activity of Dried Chemical Residues on Hard Nonporous Surfaces, with Exposure and Wear Activity after a 5 Minute Contact Time						
MRID Number	**Organism**	**Lot No.**	**Amount Recovered X 4 carriers Mean CFU**	**Mean Log Reduction**	**Mean Percent Reduction**	**Carrier Population (Log_{10}CFU/ Carrier)**
490899-19	*Staphylococcus aureus* ATCC 6538	P11130-07	0	>4.90	>99.9988%	6.08
			0			
			0.5			
			0			
		P11130-08	0	>4.90	>99.9988%	
			0.5			
			0			
			0			
		P11130-09	0.5	>5.32	>99.9995%	6.50
			0.5			
			0			
			0			
490899-19	*Enterobacter aerogenes* ATCC 13048	P11130-07	23.5	>4.74	>99.996%	6.76
			57			
			0			
			0.5			
		P11130-08	3.5	>5.24	>99.992%	
			0			
			1			
			>300			
		P11130-09	0	>4.15	>99.982%	6.73
			32.5			
			6			
			121.5			
490899-20	CA-MRSA (NARSA NRS123, Genotype USA 400)	P11130-07	0	>5.09	>99.999%	6.27
			0			
			0.5			
			0			
		P11130-08	3	>4.60	>99.997%	
			0			
			8			
			0			

Kimberly Clark Final Report for Confirmation of Initial Treatment Wetness for Residual Self Sanitizing Pre-Saturated Towelette/Wipe Product Visual Wetness Test Results at 15 seconds time period			
MRID Number	**Hitman Wipe Lot#**	**Carrier Number**	**Visual Wetness Confirmed**
490899-21	P11130-07	1	Yes
		2	Yes
		3	Yes
	P11130-08	1	Yes
		2	Yes
		3	Yes
	P11130-09	1	Yes
		2	Yes
		3	Yes

VI. CONCLUSIONS:

1. The submitted efficacy data **support** the use of the ready-to-use towelette, Hitman Wipe, as a disinfectant against the following organisms on hard, non-porous surfaces for a 5-minutes contact time with a 5% soil load:

Salmonella enterica (ATCC 10708)	MRID 490899-08
Escherichia coli O157:H7 (ATCC 35150)	MRID 490899-10
Klebsiella pneumonia (ATCC 4352)	MRID 490899-11
Streptococcus pyogenes (ATCC 19615)	MRID 490899-12

Acceptable killing was observed in the subcultures of the required number of carriers tested against the required number and age of product lots. Viability controls were positive for growth. Purity controls were reported as pure. Sterility controls did not show growth. Neutralization confirmation testing showed positive growth of the microorganisms.

2. The submitted efficacy data **support** the use of the ready-to-use towelette, Hitman Wipe, as a disinfectant against the following organism on hard, non-porous surfaces for a 6-minutes contact time (no soil load):

Staphylococcus aureus (ATCC 6538)	MRID 490899-09

Acceptable killing was observed in the subcultures of the required number of carriers tested against the required number and age of product lots. Viability controls were positive for growth. Purity controls were reported as pure. Sterility controls did not show growth. Neutralization confirmation testing showed positive growth of the microorganisms.

3. The submitted efficacy data **does not support** the use of the ready-to-use towelette, Hitman Wipe, as a disinfectant against the following organism on hard, non-porous surfaces for a 6-minutes contact time with a 5% soil load:

Pseudomonas aeruginosa (ATCC 15442)	MRID 409899-09

The required number of product lots and carriers was not tested and failed to demonstrate acceptable killing.

4. The submitted efficacy data **does not support** the use of the ready-to-use towelette, Hitman Wipe, as a fungicide against the following organism on hard, non-porous surfaces for 7 days with a 5% soil load.

Aspergillus Niger (ATCC 6275)	MRID 490899-16

Although acceptable killing was observed in the subcultures of the required number of carriers tested against the required number of product lots, disinfection claims (including fungicidal) support kill claims within ≤ ten (10) minutes contact period. Appropriate residual activity testing must be submitted and accepted for these claims. Viability controls were positive for growth. Purity controls were reported as pure. Sterility controls did not show growth. Neutralization confirmation testing showed positive growth of the microorganisms.

5. The submitted efficacy data **support** the use of the ready-to-use towelette, Hitman Wipe, as a disinfectant with virucide activity against the following microorganisms on hard, non-porous surfaces in the presence of a 5% organic soil load for a 5 minute contact time:

Influenza A virus (H1N1), ATCC VR-1469	MRID 490899-13
Respiratory syncytial virus (RSV), ATCC VR-26, Strain Long	MRID 490899-14
Human Coronavirus, ATCC VR-740	MRID 490899-15

Recoverable virus titers of at least 10^4 were achieved. No cytotoxicity was observed. Complete inactivation (no growth) was indicated in all dilutions tested.

6. The submitted confirmatory efficacy data **support** the use of the ready-to-use towelette, Hitman Wipe, as a sanitizer with bactericidal activity against the following organisms on hard, non-porous, non-food contact surfaces in the presence of a 5% organic soil load for a 15 second contact time:

Enterobacter aerogenes (ATCC 13048)	MRID 490899-17
Staphylococcus aureus (ATCC 6538)	MRID 490899-17
Staphylococcus aureus CA-MRSA (NARSA NRS123) (Genotype USA400)	MRID 490899-18

Results show a bacterial reduction of at least 99.9 percent over the parallel control within 15 seconds for the required number and age of product lots. Viability controls were positive for growth. Purity controls were reported as pure. Sterility controls did not show growth and neutralization confirmation testing showed positive growth of the microorganisms. Antibiotic resistance was demonstrated for CA-MRSA in confirmation tests.

7. The submitted efficacy data **support** the use of the ready-to-use towelette, Hitman Wipe, as a sanitizer with residual activity against the following microorganisms on hard, non-porous, non-food contact surfaces in the presence of 5% organic soil:

Enterobacter aerogenes (ATCC 13048)	MRID 490899-19
Staphylococcus aureus (ATCC 6538)	MRID 490899-19
Community Acquired Methicillin Resistant *Staphylococcus aureus* -CA-MRSA (NARSA NRS123, Genotype USA 400)	MRID 490899-20

Bacterial reduction of at least 99.9% over the parallel control was observed within 5 minutes on surfaces treated approximately 46 - 54 hours prior to testing. All lots were >60 days old at the time of testing. Neutralization confirmation testing showed positive growth of the microorganisms. Purity controls were reported as pure. Sterility controls did not show growth. Antibiotic resistance was demonstrated for CA-MRSA in confirmation tests.

8.) The submitted data demonstrated initial wetness (confirmed visually by tissue paper and analytically by gravimetric analysis) and thus met the EPA success criteria achieving an initial weight of the test substance greater than zero and visual wetness following treatment at 15 seconds (MRID 490899-21).

VII. RECOMMENDATIONS:

1. The label claims that the ready-to-use towelette, Hitman Wipe is an effective disinfectant against the following organisms on hard, non-porous surfaces in the presence of 5% organic soil for a 5-minutes contact time:

Salmonella enterica	(ATCC 10708)
Escherichia coli O157:H7	(ATCC 35150)
Klebsiella pneumonia	(ATCC 4352)
Streptococcus pyogenes	(ATCC 19615)
Influenza A virus (H1N1),	(ATCC VR-14690
Respiratory syncytial virus (RSV)	(ATCC VR-26)
Human Coronavirus	(ATCC VR-740)

These claims are **acceptable** as they are supported by the submitted data.

2. The label claims that the ready-to-use towelette, Hitman Wipe is an effective disinfectant against the following organisms on hard, non-porous surfaces (no soil load) for a 6-minutes contact time:

Staphylococcus aureus	(ATCC 6538)

These claims are **acceptable** as they are supported by the submitted data.

3. The proposed label claims that the ready-to-use towelette, Hitman Wipe is effective as a non-food contact sanitizer against the following organisms at a contact time of 15 seconds on

hard, non-porous surfaces:

Enterobacter aerogenes	(ATCC 13048)
Staphylococcus aureus	(ATCC 6538)
Staphylococcus aureus CA-MRSA	(Genotype USA 400)

These claims are **acceptable** as they are supported by the submitted data.

4. The proposed label claims that the ready-to-use towelette, Hitman Wipe is an effective 24 hour residual sanitizer against the following organisms at a contact time of 5 minutes on hard, non-porous, non-food contact surfaces:

Enterobacter aerogenes	(ATCC 13048)
Staphylococcus aureus	(ATCC 6538)

Community Acquired Methicillin Resistant *Staphylococcus aureus* -CA-MRSA (Genotype USA 400)

These claims are **acceptable** as they are supported by the submitted data.

5. The proposed label claims that the ready-to-use towelette, Hitman Wipe is an effective 7 day hard surface mildewstat against the following organism:

Aspergillus Niger	(ATCC 6275)

This claim is **unacceptable** as it is not supported by the submitted data.

6. **LABEL RECOMMENDATIONS**

- Page 2, Remove *Aspergillus niger* 7 day residual claim. The efficacy data submitted does not support the claim.
- On page 3 under to clean and disinfect: the information stating that the product will provide up to 24 hour disinfection needs to be removed – this is a residual self sanitizing claim, not a disinfectant claim.
- On page 3 under residual self-sanitizing directions: remove repeat residual self-sanitizing directions to maintain 24 hour sanitization or rewrite to indicate repeat after the 24 hour period. The residual self-sanitization claim after one application includes a 24 hours time period of residual activity.
- On label page 6, the claims "kills 99.9% of bacteria…" and "bactericidal, mildewstat, and virucidal" must be qualified to the labeled organisms.
- On page 6 left column, 9th bullet, the claim is made that the product disinfects *Enterobacter aerogenes* (ATCC 13048) and Community Acquired Methicillin Resistant *Staphylococcus aureus* -CA-MRSA (Genotype USA 400). These claims must be

removed because data was not submitted to support this claim.

- On page 6, 13th bullet on the left column, the disinfectant claims and sanitizing claims must be separated.

- Page 8, Remove "Surfaces are quickly re-contaminated". It implies heightened contamination and efficacy claims.

- Page 8, Remove "[and prevents mold and mildew for up to 7 days]. This should not be associated with a residual claim.

- Page 9, remove all claims associated with killing and preventing mold & mildew for 7 days. The efficacy data submitted does not support these claims.